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Comparative Analysis of the Anti-inflammatory Effects of E-MTA, Ketac-E, and Sealapex on Human Periodontal Ligament Stem Cells

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ABSTRACT

Background: Root Canal Treatments play a critical role in managing periapical and pulp diseases, but failure to control the inflammation may result in treatment failure. Newer developments have targeted anti-inflammatory bioactive sealers as potential contenders. The objective of this study was to explore the anti-inflammatory potential of Endoseal E-MTA (E-MTA), Ketac-Endo (Ketac-E), and Sealapex (S-apex) on cultured Human periodontal ligament stem cells.

Methods: After Study approval (BMU-EC/06-2022) from Baqai Medical University Karachi, this study was conducted from September 2022 to February 2023. Three Sealers as commercially available products were used on the cultured hPDLSCs via in-vitro experiments and treated with serial concentrations of 20ul, 40ul, and 60ul respectively to perform MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell viability assay. Expression profiling via Real Time-PCR of anti-inflammatory biometers was determined via RNA extract from 24h MTT assay due to its greater reproducibility. While data were subjected to statistical analysis (ANOVA Testing) to compare the effectiveness of each sealer using SPPS version 22 with significant p-value<0.05.

Results: E-MTA elicits a noticeably enhanced production of IL-10 (2.97 fold) and TGF-β (3.12 fold) levels than its parent compound MTA, demonstrating enhanced anti-inflammatory and immunomodulatory potential while Ketac-Endo and Sealapex showed lesser relative gene fold values.

Conclusion: It has been concluded that E-MTA exhibits more enhanced bioactivity and capacity to improve the RCT outcome due to reduced inflammation and increased tissue regeneration than Ketac-Endo and Sealapex.

Keywords: Endoseal MTA, Ketac-Endo, Sealapex, IL-10, TGF-B, Tissue Repair.

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INTRODUCTION

Oral health is a cornerstone to overall well-being and influences basic aspects of every day such as the ability to eat, speak, and engage socially. Despite tremendous advancements in dental care, conditions like periapical and pulp diseases remain global public health problems resulting in painful and poor oral health experiences[1]. To address these conditions, root canal treatment (RCT) continues to serve as a gold standard, to eradicate infections, reduce inflammation and prevent loss of natural teeth[2]. However, uncontrolled inflammation can lead to treatment failures causing recurrent infections and longer recovery times. Thus, new and more effective therapeutic strategies are urgently needed to optimize outcomes[3].

The selection of an endodontic sealer for the RCT is a crucial step for making the procedure successful. Sealers have traditionally been evaluated on how well they seal, how biologically compatible they are, and their mechanical characteristics [4]. While these parameters remain important, recent work has underscored the necessity to evaluate sealers for their capability to modulate inflammation and facilitate tissue repair. By extending the functional landscape of endodontic materials, bioactive sealers have the potential to offer anti-inflammatory and regenerative properties that could potentially enhance clinical outcomes[5]. Amongst the recent advancements, endodontic sealers such as Endoseal MTA (E-MTA), Ketac Endo (Ketac-E), and Sealapex, (S-apex) have caught a lot of attention for their biological effects on periapical tissues[6]. The unique properties of these sealers are exhibited due to the presence of primary components used in each sealer [7]. Although E-MTA displayed some favorable effects in modulating inflammatory cytokines, the role of these sealers in modulating other aspects of the immune response is not sufficiently explored[8]. Interleukins are inflammation and tissue wound repair [9,10].

TGF-β helps in tissue repair, and fibrosis and affects immune responses by inhibiting immune cells from activating. In the context of endodontic treatments, these biomarkers are both important for insights into the potential therapeutic benefits of sealers in reducing inflammation and promoting healing[11]. Human periodontal ligament stem cells (HPDLSCs) are mesenchymal stem cells derived from the periodontal ligament, known for their regenerative potential in periodontal tissue repair. Despite these progresses, very little data is available that compares the effect of E-MTA, Ketac-E, and S-apex on IL-10 and TGF-β expression. This comparison is important because it will enable clinicians to make informed choices about materials that improve biological and clinical outcomes.

The study aimed to evaluate the ability of E-MTA, Ketac-E, and S-apex to modulate inflammation and promote tissue repair by assessing the expression of IL-10 and TGF-β. This will determine which endodontic sealer has a favorable recovery profile.

METHODS

Under institutional ethics approval (BMU-EC/06-2022), from September 2022 to February 2023, this study included in-vitro experiments based on cell culture and their treatments. It was conducted in collaboration with Baqai Medical University Karachi, de Montmorency College of Dentistry, Lahore, and Karachi Medical University Pakistan possessing distinct experimental and statistical analysis responsibilities. Commercially available root canal sealers such as E-MTA, Ketac-E, and S-apex, were used and prepared as per their manufacturers' protocols. Extracted human teeth were used to evaluate the efficacy of the sealers. Only sound teeth without significant decay were selected, and they were stored in a sterile saline solution to maintain hydration and prevent contamination before the experiment. This ensured standardized conditions for testing interactions with HPDLSCs. The Human periodontal ligament stem cells HPDLSCs were obtained from the cell culture laboratory of the University of Lahore and transported in culture media with standard SOPs of incubation and growth temperature. HPDLSCs were sub-cultured in 100 mm dishes at 27°C with α-Modified Eagle medium (α-MEM) and 2-mercaptoethanol. Fresh serial dilutions in this medium were prepared for each experimental treatment before using the sealers to ensure accuracy and consistency. Stock solution was prepared and reconstituted for each sealer to give final working concentrations of 20, 40, and 60 µg/ml. These were applied to hPDLSCs plated at the density of 10,000 cells/well in 100 µL of medium in 96 well plates. Then the plates were incubated at 37°C in a humidified atmosphere with 5 % CO_2. Cell viability was measured with the MTT assay, with 10 µL of the MTT solution (10 mg/ml in phosphate-buffered saline) added to each well after 24 and 48 hours of treatment.

After treatment, total RNA was extracted from the cells and subjected to biomarker analysis. cDNA synthesis was performed using Thermo Fisher Scientific reagent protocol (AM7832) and a sample was also analyzed for RNA quality and concentration (ng/µL). IL-10 and TGF -β expression were evaluated using quantitative PCR (qPCR). The extracted RNA from the desired samples was analyzed for its purity and integrity by performing nanodrop 2000/2000c, which were observed through nanodrop software i.e. Nanodrop ND-1000 Spectrophotometer. cDNA was synthesized using a Thermo scientific kit (Catalogue # K1622, Thermo Fisher Scientific, USA), and the primers were designed on serial cloner by taking the consensus CDS sequence of required genes from NCBI. CDS consensus sequences from the NCBI database were used to create specific primers for these biomarkers.

Gradient PCR optimization was also performed to determine the best reaction conditions and the in-silico validation was performed using UCSF Genome Browser. This protocol was designed to define the differential influence of EMTA, Ketac-E, and S-apex on IL10 and TGF β expression, to ascertain their ability to regulate inflammation and promote tissue healing in periodontal ligament stem cells. Intergroup stats were inferred at p-values (<0.05 as significant) by using ANOVA (one-way) testing using SPSS version 22.

RESULTS

The primer sequences were confirmed by doing In-Silico PCR on the UCSC Genome Browser. The list of designed primers is given in (Table 1).

Table 1: Primer Sequences Selected Biomarkers

Target Gene	Primer Sequences
TGF-β	Forward: 5'GCGAATCAATGGACTCTGG3'
	Reverse: 5'GACATCTGTACCAGACCGAG3'
IL-10	Forward: 5'GAAGGAGCTGCTCTTCCGA3'
	Reverse: 5'GAGCATGACCCTGTAGGC3'

The RT-qPCR was optimized according to each primer's annealing temperatures (Tm). Conditions for the three major steps of RT-qPCR, consisting of denaturation, annealing, and extension were set according to each primer as shown below in **Figure 1.**

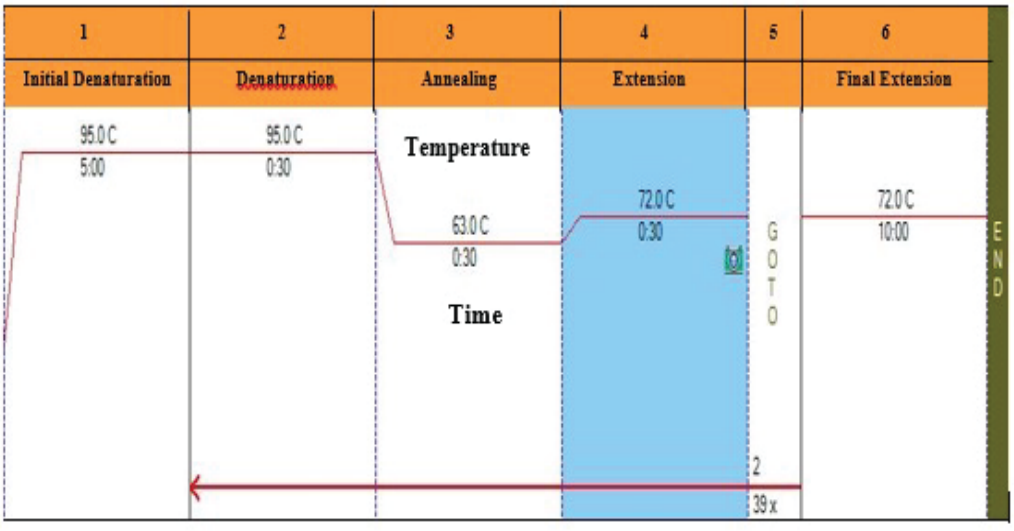

Figure 1: RT-qPCR Protocol

Both adherent, untreated HPDLSCs cells presented spindle-shaped morphology when observed under a microscope with a 10x lens. Morphological features are shown in **Fig 2(A)** Treated cells and **2(B)** Untreated Cells. Untreated HPDLSCs exhibit spindle-shaped fibroblast-like morphology with uniform alignment and consistent cell-cell linking. Treated cells often show altered morphology, such as increased spreading, stress fiber formation, and irregular cytoskeletal organization. Treatment may disrupt uniform cellular linking and affect adhesion properties by causing cytotoxicity to cellular metabolic machinery.

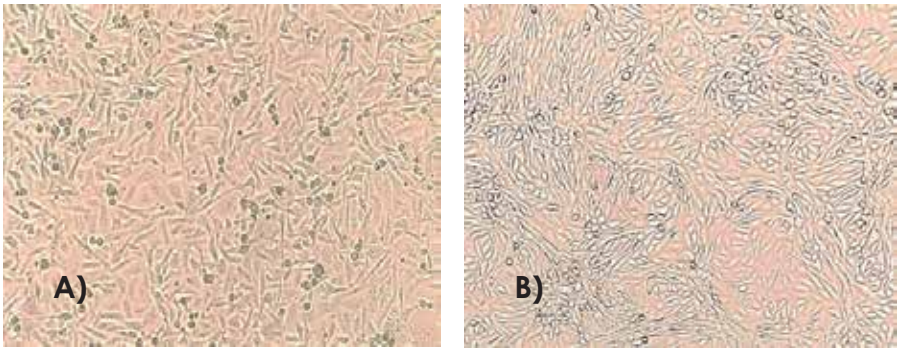

Figure 2: Microscopic Observation of Spindle-Shaped Morphology in Treated and Untreated HPDLSCs.

This study aimed to examine gene expression based on the anti-inflammatory potential of endodontic sealers when cultured HPDLSCs were treated with serial concentrations of compounds. Relative gene fold was noted as shown in (Table 2).

Table 2: Relative Gene Fold of Endodontic Materials

TGF-B		IL-10	P-value
Endodontic Sealers	Relative Gene fold	Relative Gene fold	
E-MTA	3.12	2.97	≤0.001
Ketac-E	1.7	1.8	≤ 0.05
S-apex	1.4	1.7	≤ 0.05

Our studies found that the E-MTA extract upregulated the expression of selected biomarkers. Conversely, the Ketac-E and S-apex were under-expressed compared with the cisplatin control (Relative gene fold 1.0), as shown in **Fig 3**.

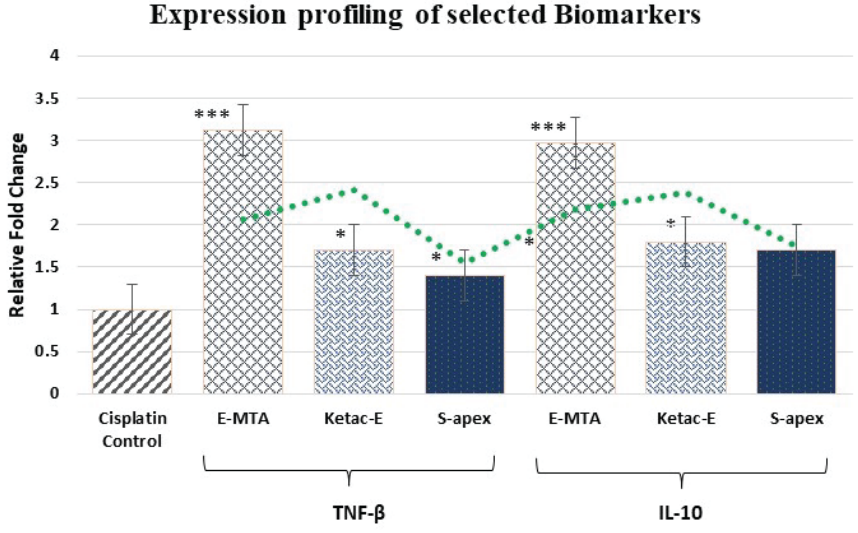

Fig:3 Expression profiling of endodontic sealers. There was a significant increase in anti-inflammatory (TNF- β and IL-10) (* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001). Expression analysis was performed based on the bio-functionality assessment of endodontic sealers and optimized by positive controls.

DISCUSSION

In this study, three commercially available endodontic sealers (E-MTA, Ketac-E, and S-apex) were evaluated for their anti-inflammatory potential These biomarkers are important for immune regulation, inflammation modulation, and tissue repair, and this biomarker framework offers a crucial framework for the bio-functional characterization of endodontic materials [12]. These findings showed that E-MTA upregulated gene expression of TGF-β (3.12 fold) and IL-10 (2.97 fold) compared to control, demonstrating superior anti-inflammatory and immunomodulatory properties. In comparison, Ketac-E showed moderate upregulation for TGF-β (1.7 fold) and IL-10 (1.8 fold) expression while S-apex demonstrated the least upregulation of 1.4-fold, and 1.7-fold for TGF-β and IL-10 respectively. These findings demonstrate the sealers' differential bioactivity and emphasize potential E-MTA clinical regimens to promote a favorable healing environment during root canal therapy[13].

TGF-β plays a double role in inflammation and tissue repair. It is a key regulating molecule that directs cellular response, inducing extracellular matrix deposition while suppressing inflammatory effects by activating immune cells and promoting tissue repair by modulating fibroblast activity[14]. E-MTA had significant upregulation of TGF-β suggesting that it can promote tissue healing processes with little inflammation, which is consistent with previous studies describing biocompatibility and bioactivity [15]. This property is of particular benefit in endodontic treatment, especially where a controlled inflammatory response is essential for the resolution of periapical lesions, and regeneration of damaged tissue [16]. IL-10 also causes a major type of inhibition that suppresses the generation of pro-inflammatory mediators and stabilizes tissues [17]. This is further highlighted by the substantial upregulation of IL-10 by E-MTA. E-MTA could effectively decrease the risk of persistent inflammation by increasing IL-10 expression. On the other hand, the relatively lower expression of IL-10 by Ketac-E and S-apex indicated the limited inflammation-suppressing potential. It therefore may impact the overall clinical efficacy of these materials [18].

Differences in composition may explain why Ketac-E and S-apex performed less in upregulating TGF-β and IL-10 than E-MTA [19]. Mineral trioxide aggregate present in E-MTA is known to be superior because of its biocompatibility and ability to release bioactive ions that interact with cellular pathways [20]. Glass ionomer-based Ketac-E sealer and S-apex containing calcium hydroxide do not display the extensive bioactive ion release characteristic of E-MTA, and may therefore be limited in their ability to influence anti-inflammatory gene expression [21]. The major determinant of the clinical effectiveness of endodontic sealers is their capacity to modulate the inflammatory process and favor tissue repair [22]. Results also suggested that bioactive sealers like E-MTA could be utilized as a material having extended functionality beyond sealing capacity, providing features of intervening in cellular responses and improving treatment outcomes. In this study, the reliability of the experimental design and outcome was further validated with the optimized use of positive controls. These findings were also consistent across replicates and indicated the robustness of the study highlighting the potential clinical implications of these results. The ability of E-MTA to dramatically upregulate TGF-β and IL-10 indicates its potential as a superior therapy which is tuned to inhibit post-treatment inflammation and enhance tissue regneration[23].

Although the results are promising, some study limitations are worth noting. The in vitro design enables a control environment for experimentation but may not replicate the complex in vivo environment [24]. The long-term effects on inflammation and tissue healing should be assessed, by validating them in animal models and clinical settings [25,26]. Finally, the molecular mechanisms that underlie the bioactivity of E-MTA by modulating TGF-β and IL-10 expression could be explored deeply. Overall, this study showed superior anti-inflammatory and bioactive properties of E–MTA versus Ketac–E and S–apex, with its enhanced activity of TGFβ and IL10 upregulation in hPDLSCs.

CONCLUSION

This study concluded that the endodontic sealers (commercial products) can affect the regulation of TGF-β and IL-10 in HPDLSCs: E-MTA is more bioactive and immunomodulatory than Ketac-E and S-apex. These studies imply that the using E-MTA might greatly improve patient benefits by accelerating the rate of tissue regeneration and decreasing inflammation in endodontic procedures. However, if these benefits are to translate into practice, several issues must be considered, including; cost, accessibility, and familiarity of the practitioners. Other studies will be required to elucidate sealer bioactivity and biomarker-based assessments to tailor the materials of choice and enhance the effectiveness in the treatment of endodontic diseases.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ETHICAL APPROVAL

The permission was obtained from the Ethical Committee of (BMU-EC/06-2022) Department of Sciences of Dental Materials, Baqai Medical University Karachi.

AUTHORS CONTRIBUTION

AI, KN conceived the idea and designed the research work, **KA, MYA, EUH** did data analysis, **MYA, NK** did the manuscript writing, **AI, EUH** did proof read and editing, All authors agreed to be accountable for all aspects of research.

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